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# Interregional traceability of Tunisian olive oils to the provenance soil by multielemental fingerprinting and chemometrics

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20 **Running title**

21 Tunisian olive oils traceability by geochemical fingerprints

## Abstract

This study aims at proving the usefulness of multielements as provenance markers of olive oils by evaluating their source, their link with the soils and their discriminating power. Eleven elements in twenty one olive oils and their paired soils from four Tunisian producing regions were characterized. The analytical results were then combined with various chemometric methods. Principal component analysis demonstrated the predominant geochemical source of the oils elements based on their associations according to Goldshmidt rule. Even though a clear correlation couldn't be proven, a correspondence was identified between the discriminating elements for both soil and olive oil represented by Fe, Rb, Mg and Pb. Linear discriminant analysis achieved classification and prediction rates of 92.1% and 87.3%, respectively. Our study shows the validity of multielements as good markers of the provenance for olive oils and that elemental fingerprinting approach can be applied to build a Tunisian olive oils database.

**Keywords:** Olive oil, Soil, ICP-MS, Geographical traceability, Geochemical fingerprints

## Chemical compounds studied in this article:

Sodium (PubChem CID: 5360545); Magnesium (PubChem CID: 888); Iron (PubChem CID: 23925); Zinc (PubChem CID: 32051); Strontium (PubChem CID: 104798); Manganese (PubChem CID: 23930), Barium (PubChem CID: 104810); Lead (PubChem CID: 5352425); Vanadium (PubChem CID: 5460753); Rubidium (PubChem CID: 105153).

## 1. Introduction

Olive oil production in Tunisia has increased due the development of modern orchards, intensification of the traditional ones and expansion into new crop producing areas, making it the second largest producer after the European Union (IOC, 2017). Besides this, Tunisian olive orchards are characterized by rich genotypic heritage approximating seventy olive tree varieties each having unique attributes (Laroussi-Mezghani et al., 2015). Yet, this key food product suffers from a low international recognition since about 99% of Tunisia's olive oil has traditionally been exported in bulk with relatively cheap price (Kashiwagi, 2012).With a growing international competitiveness, Tunisian authorities have aimed at increasing the bottled olive oil exports by the establishment in 2006 of the Fund for the Promotion of Packaged Olive Oil (FOPROHOC) and developing new products based on the organic production certification in order to enhance the recognition of Tunisian olive oil and to reach new markets such as Japan (Kashiwagi, 2012). Despite these efforts, exports of packaged olive oils are still far from the targets set of 20% (IOC, 2017). To solve this problem and to push Tunisia's olive oil producers to export less oil in bulk and more bottled and branded products, development of differentiated olive oil products such as geographical indications (GI) labels is highly recommended to introduce a product with a higher added value and fit with consumers' preferences in a highly competitive market. Therefore, Tunisia is aiming at the high-end market, seeking to enhance its competitiveness. In 2008, a study conducted by the Agency for the Promotion of Agricultural Investments (APIA) has identified 21 areas eligible for obtaining the registered designation of origin qualification. This selection is based on the differenciation of the areas according to variety, soil, climate and the know-how, but it does not take into account the physico-chemical characteristics of the oils (Laroussi-Mezghani et al., 2015).

During the previous decade, intensive research efforts have been carried out to develop methodological approaches to characterize and prove the uniqueness of Tunisian olive oils according to their geographical and/or varietal origin. Most of these efforts with a view to selecting the suitable geographical marker, focused on its molecular and biochemical composition and sensory profile. These two later parameters are highly dependent on the storage conditions of olive oils (Gomez-Alonso, Mancebo-Campos, Desamparados-Salvador & Fregapane, 2007; El Haouhay et al., 2018); hence their use as geographical markers appears unsafe. In addition to that, molecular markers are more appropriate to check for the varietal origin rather than the geographical origin (Adamo et al., 2012). An alternative and reliable marker of the provenance is therefore needed.

Numerous studies have shown that element patterns, in combination with multivariate statistics, can be successfully used to identify the geographical origin of foodstuff in general (Ariyama, Nishida, Noda, Kadokura & Yasui, 2006; Greenough, Fryer & Mallory-Greenough, 2010) and olive oil in particular in specific geographical contexts (Jimenez, Velarte, Gomez & Castillo, 2004; Benincasa, Lewis, Perri & Sindona, 2007; Camin et al., 2010a; Camin et al., 2010b; Cabrera-Vique, Bouzas & Oliveras-Lopez, 2012; Beltrán, Sanchez-Astudillo, Aparicio & Garcia-Gonzalez, 2015). These studies have laid the foundation for testing the suitability of multielements as potential provenance marker within the Tunisian geographical and geological context.

The success of multielements as geographical markers is essentially due to the fact that food elemental profile reflects its complex interaction with the environment including climate, rock mineralogy, elements mobility and bioavailibity in soils and physiological aspects typical of the specie from which it is derived (Giaccio & Vicentini, 2008). And even though the composition of olive oil could be affected by cultivation practices, extraction process and/or environmental pollution, it has been suggested that the selection of suitable elements that

reflect the geogenic origin rather than athropogenic origin such as alkaline metals especially Rubidium (Rb) and Cesium (Cs), being easily mobilized in the soil (Kelly, Heaton & Hoogewerff, 2005), or Lithium (Li) and Rb that don't suffer the influence of technological processing (Giaccio & Vicentini, 2008) can solve this issue. It is therefore necessary to check for the possible origin of the elements contained in the Tunisian olive oil.

Apart from being proved successful in other countries, elemental fingerprinting can give valuable information regarding the quality of olive oils expressed as the Maximum Residue Limit which is a quality attribute defined by the international olive council fixing the maximum allowable concentration of some toxic heavy metals (IOC, 2016).

Even though multielements (major, minor, trace and rare earth elements) were extensively used to relate olive oils to their provenance, information on how the natural factors of the provenance, including the geochemical factors, correlate with the elemental profile of olive oil is very scarce (Beltrán et al. 2015) unlike other tracers, for instance the stable isotope ratios of light elements, whose variability in olive oils according to origin was correlated with climatic and geographical parameters (Chiocchini, Portarena, Ciolfi, Brugnoli & Lauteri, 2016).

In order to prove the usefulness of the multielement fingerprint as a tool for origin authentication, the relationship with the provenance environment should be prooved. Firstly, the source of the elements in olive oil should be evaluated whether conditioned upon geochemical or anthropogenic factors. Secondly, the strength of the relationship between the geochemistry of orchard soil and elemental composition of olive oil needs to be quantified.

The present work constitutes a pilot study within a research project aiming at identifying the origin of Tunisian olive oils using chemical markers. The objectives of the present study were to:

(i) characterize the elemental profile of Tunisian olive oils from four geographical origins,

(ii) check the quality of the oils based on the maximum residue limit criteria,

(iii) verify the source of the elements in the Tunisian oils based on their associations,

(iv) evaluate the ability of multielements analysis in classifying and predicting the geographical origin of Tunisian olive oils,

v) study the link between the chemical compositions of olive oils and their provenance soils.

To the best of our knowledge, no previous study on Tunisian olive oil have ever used the geochemical fingerprints (i.e. major and trace elements) to trace back its geographical origin. The only available multi-element data concerned olive oils obtained from trees irrigated with treated wastewater in one limited geographical area (Benincasa et al., 2012).

## 2. Materials and methods

### *2.1. Sampling areas*

The olive oils used in this study originated from four producing regions namely Sfax (2 sites) and Monastir (5 sites) in the center-east and Gafsa (5 sites) and Medenine (2 sites) in the south of Tunisia. These regions are considered important olive oil producing areas whose considerable amount of their production is destined for exportation. The climate in these regions varies from arid to semi-arid Mediterranean. During the time period comprised between 1991 and 2015, the average annual temperature and rainfall for the four regions was as follows: Gafsa: 20.4°C, 194.3 mm/year; Medenine: 21.2°C, 215.8 mm/year; Sfax: 20°C, 260 mm/year and Monastir: 20.7°C, 346.5 mm/year (World Bank Group, 2018).

From a geological point of view, the outcrops in the sampling areas are indicated in Fig. 1. Gafsa samples were located on recent and actual (Holocene) sandy alluvial sediments and sand dune sediments. For Sfax samples, the first site was located on old alluviums that are

mostly aeolian sand-loam with limestone concretions and gypsum crusts (Middle-Late Pleistocene); the other sampling site was located on Mio-Pliocene continental sediments made of conglomerates and sandy clays. The sampling sites of Monastir samples were located on the same old alluviums and Mio-Pliocene sediments of Sfax sites. Medenine samples originated from a site lying above the same old alluviums and Villafranchian - lower Pleistocene conglomerates and carbonate crusts (Cohen, Schamel & Boyd-Kaygi, 1980; Sghari, 2014).

### 2.2. *Olive and soil sampling*

Only varietal olive fruits were hand-picked from three representative trees for each sample. These were autochthonous Tunisian varieties like Chemleli Sfax, Chemleli Sahli and Chemchali of Gafsa and introduced foreign varieties like Spanish Arbosana, Italian Frangivento and Greek Koroneiki (Table S1). The introduced varieties were mostly from Gafsa where they are extensively cultivated in new intensive systems compared to other regions. The sampling followed other criteria so as to sample paired olive fruit and soil samples from the same orchard to allow evaluating the relationship between provenance soil and olive oil compositions. Top and subsoil samples were collected from two layers at the depths of 0-30 and 30-60 cm using hand auger. Soil samples were taken from under each sampled tree and then mixed by weight to form a representative sample of each sampling site at each depth. Fig. 1 shows the locations of the total fourteen sampling sites recorded by a handheld GPS receiver (eTrex Legend HCx, GARMIN, UK). All samples were collected in polyethylene plastic bags and rapidly transported to the laboratory for preparation to the chemical analyses. Sampling was conducted from November to December 2015.

### *2.3. Samples preparation and analyses*

#### *2.3.1. Olive oil samples*

##### *2.3.1.1. Oil extraction*

Olive oil was extracted in Sfax Olive Institute from 1.5 kg of whole washed olive fruits by mechanical means alone. A laboratory scale 2 phases oil mill was used following the standard methods employed in industrial olive oil mills. This method consists of crushing, malaxation for 30 minutes at ambient temperature, centrifugation without addition of water and finally natural decantation. This process yields an oily phase and a very wet pomace phase. Olive oil samples were stored in amber glass bottles at 4 degrees Celsius until major and trace elements determinations at the Advanced Analysis Center, National Agriculture and Food Research Organization (NARO) in Japan.

*2.3.1.2.Digestion*

Mineralization of olive oil samples was carried out according to the method of Llorent-Martinez, Fernandez-de Cordova, Ortega-Barrales and Ruiz-Medina (2014) with minor modifications. The method consists of weighing 0.5 g of sample vigorously shaken directly into the digestion vessel and adding 7 ml of 61% electronic-grade (EL) nitric acid $HNO_3$ (Cica-Merck Kanto Chemicals, Japan) and 1 ml of 30% atomic absorption spectrometry-grade hydrogen peroxide $H_2O_2$ (Wako Pure Chemical Industries, Japan) for ultratrace analysis. The vessels were placed in a microwave digestion system (ETHOS 1600 Advanced Microwave Labstation, Milestone Inc., Italy). The program of the microwave consisted of a ramp of 15 minutes to reach 200°C and 1000 W that were maintained for additional 15 minutes. After cooling down to room temperature, samples were transferred into DigiTUBES that have an ultra-low leachable metal content, of class A tolerance at the 25 ml graduation (DigiTUBES, SCP Science, Canada) and diluted to volume with ultrapure water (Milli-Q Integral 3, Nihon Millipore, Japan). Samples were filtered using a 0.20 µm pore size syringe filter (Captiva econofilter, Agilent Technology, USA). Vessels were cleaned using the same microwave operating program after each digestion batch and rinsed with Milli-Q water. The use of glassware was avoided to prevent metal release.

187 *2.3.1.3. Quality control*

188 Confidence in measurement results is important for food analysis especially when it comes to
189 a geographic marker that will be used to check for authenticity. Evaluating reliability of trace
190 elements measurement results in olive oil is hindered by the absence of a matrix matching
191 certified reference material. The accuracy of the method was therefore evaluated using a
192 multielement oil standard S23-100Y of 100 ppm concentration (SPEXCertiPrep, USA). The
193 limit of detection (LOD) and limit of quantification (LOQ) of each element were calculated as
194 three and ten times the standard deviation of the concentrations determined in 9 independent
195 method blank samples, respectively. The method blank sample was prepared using Conostan
196 75 cSt blank oil (SCP Science, Canada) to substitute olive oil sample in the extraction step.
197 Each olive oil sample was digested and measured three times to ensure precision. Each
198 digestion batch contained one method blank to monitor contamination. Influence of
199 instrumental drift was corrected by using 10 µg $L^{-1}$ internal standard solution of Sc, Y, In, Tb,
200 Ho and Bi prepared from 10 mg $L^{-1}$ CLISS-1 standard (SPEX CertiPrep, USA).

201 *2.3.1.4. Calibration*

202 External calibration curves were built on eight different mass concentrations. Standard
203 solutions were prepared by diluting in 5.0% w/w nitric acid matrix a multielement solution
204 (XSTC-622B) containing Na, Mg, V, Fe, Mn, Zn, As, Rb, Sr, Ba, Pb of 10 mg $L^{-1}$
205 concentration (SPEXCertiPrep, USA). The concentration range for all the elements was 0-50
206 µg $L^{-1}$.

207 *2.3.1.5. ICP-MS measurements*

208 The quantification of the elements was carried out with an ICP-MS instrument (Elan DRC-e,
209 Perkin-Elmer SCIEX, Canada). Oil samples were introduced by means of a borosilicate glass

nebulizer. The ICP torch was a standard torch (Fassel type torch) with ceramic injector. ICP-MS is known to suffer from unwanted polyatomic isobaric interferences. Therefore, the elements had been monitored in standard and $CH_4$ dynamic reaction cell (DRC) modes to check for and reduce polyatomic interferences and the appropriate isotopes were used. Instrument performance was checked by a midrange continuing calibration verification (5 µg $L^{-1}$) every ten samples.

The operating conditions and parameters of ICP-MS were as follows: Rf power, 1100 W; plasma Ar flow rate, 15 L $min^{-1}$; auxiliary Ar flow rate, 1.3 L $min^{-1}$; nebulizer (carrier gas) flow rate, 0.77 L $min^{-1}$; sampler and skimmer cones, nickel; lens voltage, 7.5 V; analog stage voltage, −1700 V; pulse stage voltage, 950 V; discriminator threshold, 70 V; quadrupole rod offse,t -1.5 V; detector, dual; speed of peristaltic pump, 20 rpm; sweeps/reading, 20; replicates, 3; dwell time, 50 ms; scan mode, peak hopping; in DRC mode CH4 reaction gas flow, 0.6 L $min^{-1}$; rejection parameter *a,* 0 and Rejection parameter *q,* 0.65. Monitored isotopes: $^{23}Na$, $^{24}Mg$, $^{51}V$, $^{56}Fe$, $^{55}Mn$, $^{66}Zn$, $^{75}As$, $^{85}Rb$, $^{88}Sr$, $^{138}Ba$, $^{208}Pb$.

*2.3.2. Soil samples*

Soil samples were dried in a thermostatic oven at 40°C for 24 hours to remove moisture as a pre-treatment step prior to subsequent analyses. Major and trace elements in soil samples were measured at Geological Survey of Japan (GSJ) of National Institute of Advanced Industrial Science and Technology (AIST) in Japan following a method developed by the GSJ. It consists of preparing high dilution-ratio fused glass beads for X-Ray Fluorescence (XRF) determination of major element oxides **(**Ejima, Kon, Kawano & Araoka, 2018**)** and using the same beads for trace elements measurement by femtosecond ultraviolet Laser Ablation ICP-MS (LA-ICP-MS) **(**Kon & Hirata, 2015**)**.

*2.3.2.1. XRF analysis*

The glass beads were prepared by mixing 0.5 g of powdered soil sample with 5.0 g of lithium tetraborate flux. The mixture was heated to 1200°C during 10 minutes in a 95% Pt- 5% Au crucible using a semi-automatic fusion device (HAG-M-HF, Herzog, Germany). Four major elements in soil samples (Fe, Mn, Mg and Na) were quantified by XRF instrument (ZSX Primus III+, Rigaku Corp., Japan) with an Rh tube. The calibration lines of each element were prepared by using 14 GSJ geochemical reference samples (JA-1, JA-2, JA-3, JA-1a, JB-3,JB-1b, JG-2, JG-3, JG-1a, JGb-1, JGb-2, JR-1, JR-2 and JR-3) with their chemical compositions varying from mafic to felsic (Imai et al., 1995). The accuracy was verified each day with two reference samples (JB-1b and JG-3). The analytical uncertainties of each element were better than 1.5% as estimated from the long-term reproducibility of measurements of JB-1b.

*2.3.2.2. LA-ICP-MS analysis*

To obtain reliable abundance data for seven trace elements in the soil samples, we used a quadrupole ICP-MS system (Agilent 7500cx, Agilent Tehnologies Japan Ltd., Japan) coupled with a 260 nm (UV) Titanium-Sapphire femtosecond laser-ablation system (IFRIT, Cyber Laser Inc., Japan). The Laser ablation was operated using a crater size of 20µm, a pulse energy of 10 J $cm^{-2}$, an emission repetition rate of 1000 Hz and a pulse duration of 150 s. A galvanometric optical scanner was employed to minimize elemental fractionation and for effective ablation of the glass beads with a rastering speed of 10,000 µm $s^{-1}$ in 400 µm x 400 µm rastered area. The plasma parameters of the ICP-MS instrument were optimized to minimize the production of $^{232}Th^{16}O^{+}$ ($^{232}Th^{16}O/^{232}Th$) and the doubly charged ion interferences ($^{103}Rh/^{206}Pb$) and to maximize the intensity of $^{7}Li$, $^{29}Si$, $^{89}Y$, $^{139}La$, $^{208}Pb$ by using the NIST SRM 610 glass standard. To quantify the concentration of each element, GSJ geochemical reference samples (JP-1, JB-1b, JB-2, JB-3, JA-1, JA-2, JA-3, JR-1, JR-2, JR-3, JGb-1, JGb-2, JG-1a, JG-2, JG-3) were used to build the calibration curves. Calibration lines were established by plotting Li normalized signal intensities against the reference values of

259 the standards. The operating conditions and parameters of ICP-MS were as follows: Rf power,
260 1600 W; cool gas flow rate, 15 L $min^{-1}$; auxiliary gas flow rate, 1 L $min^{-1}$; carrier gas flow
261 rate, 0.8 L $min^{-1}$; scanning mode, peak jump; analysis mode, time-resolved analysis;
262 integration time, 240 s/ sample; dwell time, 10 ms for Li, 20 ms for V, Cr, Rb, Sr, Ba and Pb
263 and 40 ms for other elements; sweep time, 1,6 s; detector mode, analog for Li and P/A mode
264 for other elements. Isotopes measured: $^{6}Li$, $^{51}V$, $^{66}Zn$, $^{75}As$, $^{85}Rb$, $^{88}Sr$, $^{137}Ba$, $^{208}Pb$. The
265 repeatabilities were better than 5% for V, Rb, Sr, Pb and Ba; and 9% for Zn and As (Kon &
266 Hirata, 2015).

267 2.4. *Data analysis*

268 The approach used in this study to reveal related features and samples distribution in the olive
269 oil and soil datasets according to geographical provenance included uni- and multivariate
270 statistical algorithms. The analysis of variance (ANOVA) is a parametric test used to
271 determine the statistical effect of geographical origin on the multi-elemental profile of the
272 different groups based on the variability of their means. After checking for normality of
273 distribution and homogeneity of variance assumptions in the oil dataset, the non-parametric
274 Kruskal-Wallis test was applied to detect location shift in the distribution. The unsupervised
275 dimension-reducing Principal Component Analysis (PCA) procedure creates a new feature
276 space defined by uncorrelated principal components that are linear combinations of the
277 original variables, to reduce redundancy. PCA with Varimax rotation was applied to assess
278 the multi-element associations in the oil dataset and reveal the underlying processes
279 responsible for such associations. The Linear discriminant analysis (LDA), a supervised class
280 modeling approach, was applied to evaluate the multielements performance to classify the
281 olive oils and soils according to their origin providing a way for validating the predictive and
282 generalization ability of the model. LDA maximizes the ratio of between class variance to
283 within class variance based on a defined classification. The sensitivity of the established

LDA models was evaluated using the leave one out cross-validation (LOOCV) technique considering the relatively small number of cases. Spearman's rank correlation coefficient was calculated to evaluate the relationship between soil and olive oil chemical composition. Kruskal-Wallis, PCA, LDA and Spearman's correlation were performed using IBM SPSS Statistics software for Windows, version 24.0 (IBM Corp., Armonk, N.Y., U.S.A.).

## 3. Results and discussion

### *3.1. Quality control*

As it can be seen from table 1, accuracy results were in the range 84–102% for almost all the elements measured in the oil standard except for Mg that gave 66%. All of the reported elements had concentrations 100% greater than LOD except for Zn and Pb whose concentrations were 95% and 32% greater than LOD, respectively. As for LOQ, the results were less satisfactory and only Na, Rb and Sr were 100% measured with quantifiable amounts in olive oil samples. The rest of the elements presented concentrations comprised between 0% (Pb) and 76% (As) higher than the LOQ. The linearity was satisfactory with $R^2 \geq 0.999$ for all the elements. For the statistical treatments, we used the original concentrations of all of the 11 elements (Table 1) even those with values below the LOD and/or LOQ.

### *3.2. Elemental profile of olive oils*

Table 2 shows descriptive statistics of detected elements in olive oil samples from the four geographical origins, all cultivars taken together. As expected, Na and Mg were the most abundant elements followed by Fe, Zn, Sr, As, Mn, Ba, Pb, V and Rb. As most of Tunisia's bedrocks are of sedimentary nature, Na, Mg and Fe are found in high concentrations in soils of different types (Table 4). Therefore, finding them as major elements in olive oils as well testifies to their absorption by olive trees from soil in higher proportions compared to micronutrients and trace elements.

The elemental profiling of olive oils can give valuable information about their quality expressed as total content of specific elements. A quality criteria known as Maximum Residue Limits (MRL) was established by the International olive council (IOC, 2016) fixing maximum levels of metal ions; such as As and Pb to 100 µg $kg^{-1}$ and Fe to 3000 µg $kg^{-1}$; known to increase the rate of oil oxidation. All of the olive oil samples had a concentration of Fe, As and Pb far below the maximum limits which shows the good quality of Tunisian olive oils.

Since data on Tunisian olive oil are practically inexistent, concentrations found in the present work were also compared to concentrations measured in olive oils from several Mediterranean countries (Table S2). The reviewed literature showed a wide variability in the concentrations of many elements even at the level of the same country. Our results agreed the most with those on Tunsian olive oils from Sfax region (Benincasa et al., 2012), Italian Olive oils from southern and central Italy (Benincasa et al., 2007) and Croatian olive oils (Zeiner, Steffan & Juranovic-Cindric, 2005; Juranovic-Cindric, Zeiner & Steffan, 2008; Zeiner et al., 2010) but differed significantly from other results for the majority of elements. Concentrations of Na found in this study were in the range 1.6-4.3 mg $kg^{-1}$ similar to Sfax olive oils (4.6 mg $kg^{-1}$) reported by Benincasa et al. (2012) and to Croatian olive oils (3.4 mg $kg^{-1}$) reported by Zeiner et al. (2010) but 50 times lower than Portuguese oils (Gouvinhas et al., 2016) and 1000 times higher than Spanish oils (3.8-7.8 mg $kg^{-1}$) (Beltran et al., 2015). The data of Mn in the present study ranged between 12-18 µg $kg^{-1}$ and was 10 times lower than in Sfax oils (Benincasa et al., 2012), within the range of Italian olive oils from five different geographic regions of central and southern Italy (Rossano, Andria, Lamezia, Spoleto and Pescara) (n.d.-25 µg $kg^{-1}$) (Benincasa et al., 2007) and about 100 times higher than Italian olive oils from Sicily and Tuscany (< 0.20 µg $kg^{-1}$) (Camin et al., 2010a). Mn concentrations also varied from 100 times higher to 1.8 times lower than Mn quantified in Spanish olive oils. If this variability had been solely due to the geochemistry of the provenance soil, then this

evidence would further prove the usefulness of elemental fingerprinting. However, we presume that such variability could have been partially affected by the use of different analytical methods for elements quantification in olive oils. As previously proved in a comparative study, significant differences were found between element concentrations after different pre-treatment procedures (Bakircioglu et al., 2013) and this variability was suggested in another study (Kara, Fisher & Hill, 2015). This situation highlights the need for a standard official method of multielements quantification in olive oils for the purpose of geographical traceability to make rigorous comparisons possible.

The nonparamtric Kruskal-Wallis test was applied to evaluate the difference between the four origins since data failed the normality of distribution and homogeneity of variance assumptions assessed through Shapiro-Wilk and Levene's tests respectively. Mn and Zn were significantly different among three pairs of origin ($p < 0.01$) (Table 2). Nine out of eleven elements showed significant difference between the mean ranks of at least one pair of origin ($p < 0.05$) demonstrating that oils from different regions have a characteristic elemental profile. There was no evidence of difference in the concentrations of V and Fe ($p > 0.05$) between any pair of the four origins. Among all origins, the highest concentrations were found in oils from Gafsa and Medenine except for Rb, Ba and Pb that had higher concentrations in Monastir and Sfax oils. As pointed out in a previous study on wine traceability, the soil and dust are probable source of Ba and Sr in food, so variations of Ba, Rb and Pb found in olive oils from different origins; in this case Monastir and Sfax versus the rest of the origins; can be explained by differences in regional soil geochemistry (Fiket, Mikac & Kniewald, 2011).

### *3.3. Multivariate analyses*

#### *3.3.1. PCA*

Univariate methods alone are insufficient to get a global view with regards to elements discriminating potential taking into consideration the complex interactions between the different elements. Multivariate approaches such as PCA can help understanding the relationship between the predictors. PCA can be useful in depicting elements associations and evaluating whether such associations are conditioned upon natural or anthropogenic processes. In our study, PCA was applied to the correlation Matrix with Varimax rotation and four components were extracted explaining 75% of the original variance in the dataset. Examination of the component loadings (Table 3) shows that Rb, Mg, Sr and Ba loaded high in the first component that explained 27% of the overall variance. Bearing in mind that strong associations of the elements in olive oil reflect similar behaviour or bioavailability from the soil, the first component represents elements grouped according to Goldshmidt's geochemical classification (Greenough et al., 2010). Rb, Sr, Mg and Ba are classified as lithophile elements and occupy the first two groups of the periodic table which displays elements with similar chemical characteristics such as solubility. Taking into account Kruskal-Wallis test results, it is interesting to note that the elements of the first PC were found in higher concentrations in Monastir and Sfax olive oils except for Mg and Sr. Thus it appears that this component groups the elements that also show significant variation between Monastir and Sfax and the rest of the origins. This occurrence can be related to the composition of the soils on which the olive trees were grown. As shown on the geological map, samples originating from Monastir and Sfax were located on the same mixture of conglomerates sand and clay. Shales are known to accumulate rare elements such as Rb and Ba (Krauskopf, 1995). The second component was dominated by Mn, Fe, Zn and Na. Jimenez et al. (2004) mentioned that the relationship between Mn and Alkali elements and soil has been proven and used in geographical traceability issues. On the other hand, Mn and Fe have very similar geochemical behaviour and are common in Fe-Mg silicates and carbonates given the similar ionic radius of Mn, Fe,

382 Mg and Ca that favours substitutions in minerals (Pohl, 2011). Similarly to what was
383 previously mentioned for the first PC, the elements grouped in the second PC were more
384 concentrated in Gafsa olive oils, derived from olives located on sandy soils rich in silicates,
385 and showed a significant difference between Gafsa and the rest of the origins except for Fe.
386 The elements distribution in the third and fourth component is less clear and didn't allow us to
387 determine the predominant cause of the associations. The elements grouping in PCA suggests
388 that olive oils elemental profile is substantially shaped by geochemical processes. This agrees
389 with findings of Ariyama et al. (2006) on element contents in Japanese onions and Greenough
390 et al. (2010) on Canadian Maple syrups and wines where a tendency for elements of the same
391 group in the periodic table or from similar geochemical groups to form clusters was related to
392 similar behaviour of elements in the foodstuff in question. This information supports the
393 utility of using multielements for olive oil traceability.

394 3.3.2. Classification and prediction

395 PCA provides evidence that could only indicate the elements behaviour. In our case, PCA is
396 inappropriate to check the data points pattern based on a bidimensional plot since the variance
397 explained by the first two components was deemed too small to successfully substitute the
398 original dimensions without loss of information. In order to gain information on the
399 classification potential of multielements, LDA was applied to 63 cases (21 samples three
400 times replicated to allow the groups of small size to have a reasonable ratio of cases to
401 variables) organized in four groups corresponding to the four origins and 11 independent
402 variables. The LDA calculated three canonical linear discriminant functions where the first
403 two accounted for 98% of the total variance. A total of 92.1% of the original samples were
404 correctly classified to their origin. The bidimensional plot (Fig. 2a) shows that olive oils from
405 Gafsa, Medenine and Monastir can be differentiated on the basis of the first function. Sfax
406 and Monastir samples cannot be clearly separated on the basis of the first function but are

well differentiated along the second function. The analysis of the standardized coefficients indicated that Ba (-1.1), Rb (0.70), Mg (0.53) and Zn (0.45) had the largest absolute values, i.e. the greatest discriminating ability, in the first function while Fe (0.84), Pb (0.74), As (0.52) and V (0.47) showed the largest absolute values in the second function. Indeed, the first set of variables (Ba, Mg and Zn especially) had significant differences between Gafsa and the rest of the oil samples while Pb, featured in the second function, had a significant difference only between Sfax and Monastir olive oils. To verify the predictive and generalization power of the model, LOOCV was applied and 87.3% of the samples were correctly predicted. The lower prediction rate was probably due to the small number of samples used to train the model but the sensitivities achieved are still considered satisfactory. The fact that olive oils from the same origin plotted together; which indicates that they are chemically similar; demonstrates the predominant influence of provenance on olive oils composition. This composition can considerably be attributed to the geochemistry of soils as we showed in the PCA analysis. The homogeneity of soils in Gafsa, mostly sandy, could have played a positive role in achieving high classification rate despite that various cultivars came from that region which again proves the importance of soil composition. However, olive oils from Sfax, Monastir and Medenine were mostly from the same cultivar within the same origin which could also contribute to the classification success achieved if the elements profile would depend on the cultivar as well. At this point it is important to evoke that Beltran et al. (2015) reported that classification of south-western Spanish olive oils to their municipality of origin was not influenced by the cultivars. In another study on olive oils from central and southern Italy, Benincasa et al. (2007) showed that the difference induced by the origin was more significant than by cultivar.

#### 3.3.3. Influence of cultivar

In order to test this hypothesis, we applied LDA using this time 9 groups corresponding to each single cultivar and origin. This hypothesis can be verified mainly based on Koroneiki olive oils coming from Gafsa and Monastir since other cultivars were sampled from these origins. The LDA calculated 8 canonical linear discriminant functions where the first two accounted for 77% of the total variance. A total of 97% of the original samples were correctly classified to their origin. The analysis of the standardized coefficients indicated that Na (-1.4), Sr (-1.33), Ba (-1.05), Mn (-1.04) and Fe (0.98) had the largest absolute values in the first function while Zn (0.87) and Mg (0.83) had the largest absolute values in the second function. The bidimensional plot (Fig. 2b) shows that olive oils from the same origin tend to plot next to each other even if the cultivar difference was taken into account. This explains the short distance on the plot between Koroneiki from Gafsa (100% correct classification) and other cultivars from Gafsa compared to that between Koroneiki from Gafsa and Koroneiki from Monastir (100% correct classification). This observation is further confirmed by LOOCV results were 100% of Koroneiki from Monastir olive oils samples were ascribed to their correct origin and Koroneiki from Gafsa samples were attributed to olive oils from Gafsa with some misclassifications though (56% Koroneiki Gafsa, 22% Chemlali Gafsa, 11% Frangivento Gafsa and 11% Zalmati Medenine) but 89% of the samples still can be classified as coming from Gafsa. These results agree with the findings of Benincasa et al. (2007) and Beltran et al. (2015). In future work, sufficient data on coexisting cultivars in different regions need to be collected that one may further confirm that trend.

### *3.4. The link between soil and oil*

The key point of using multielements in geographical traceablilty matter relies on the hypothesis that soil chemistry is the main factor that influences that of olive oil. Accordingly, it is relevant to check the strength of link between olive oil and soil elemental composition. Table 4 shows the concentration of elements in soil samples of the four origins and at the two

depths. Welch's test, an equivalent to ANOVA used when group variances are not equal, was employed to test the differences between the two depths and the four origins. The element concentrations didn't show any significant difference between the two depths but all elements had a significant difference between at least two origins. Spearman's rank correlation coefficients were calculated for elements in olive oils and soils taken from the two layers. In the group of the 11 determined elements, only Zn exhibited a statistically significant correlation between its content in olive oil and in soil no matter what the layer depth is but the correlation sign was negative: (c.c. = -0.62, p = 0.019 for 0-30 cm) and (c.c. = -0.74, p = 0.0030 for 30-60 cm). As for the 0-30 cm layer, Pb (c.c. = -0.58, p = 0.031) showed statistically significant correlations between its content in olive oil and soil. The comparison of soil and oil concentrations shows that Fe, Mn, Zn and Na and Pb had the highest concentrations in soils of Monastir and lower concentrations in soils of Gafsa. Contradictorily, these elements had higher concentrations in Gafsa olive oils compared to Moanstir. Thus, the significant negative correlation coefficient obtained for Zn and Pb probably means that their content in olive oils depends not only on the element contents in soils but also on other determinants with the closest related factor being climate (Greenough et al., 2010). In fact, Gafsa is located in a middle arid bioclimatic zone characterized by higher temperatures and evapotranspiration rates favouring higher water and elements uptake as opposed to Monastir situated in a lower semi arid climate marked by lower temperatures and higher precipitations (Kefi, Dat Pham, Kashiwagi & Yoshino, 2016). The other elements didn't show any significant correlation between soil and olive oil which could be explained by many reasons: (i) the use of total elements concentrations rather than bioavailable fraction of elements in soil, (ii) elemental fractionation during olive oil extraction process, (iii) physiological aspects of the cultivars that impose a specific elemental uptake pattern and partitioning within the

different olive tree tissues and differences in climate conditions which affect water and elements uptake by olive trees might be plausible explanations.

Even though most of the elements didn't show a significant correlation, differences in olive oil composition need to be checked whether they can be explained by differences in soil multielemental composition. When stepwise LDA analysis was applied to the soils elemental composition, soil samples were 100% correctly classified and 97% validated to their origin including the two depths and showing less scattering than olive oil samples (Fig. 2c). The analysis of the standardized coefficients of the first two functions out of three calculated functions indicated that Fe (6.4), Rb (-5.06), Mg (-4.37), Sr (2.52) and Pb (1.85) had the largest absolute values in the first function while Mn (4.61) had the largest absolute value in the second function. Thus, Fe, Mg, Sr, Rb, Mn and Pb were selected by the stepwise LDA as the most discriminating elements. This shows some agreement with olive oil discriminating elements and revealing Fe, Rb, Mg and Pb as common discriminating elements between olive oils and their provenance soils.

## 4. Conclusion

The results obtained in this study provide an evidence to the validity of multielements as good markers of the provenance for olive oils. The predominant geochemical origin of the elements contained in olive oils attests to their link with the provenance soils. Amongst the analyzed elements, only Zn exhibited a significant correlation coefficient indicating that the soil total concentration is not the best predictor of the oil elemental composition. Even though a quantitative estimation of the strength of the relationship between soil and oil total elemental concentrations was not possible for most of the elements, a correspondance between the discriminating elements was identified represented by Fe, Rb, Mg and Pb as revealed by LDA. This shows that the soil's geochemical signature has been transferred to the oil with some alterations though.

Another important finding of this study is the good quality of the analyzed oils based on the MRL quality criterion as Fe, As and Pb had concentrations far below the maximum limit. The LDA proved the good inter-regional discrimination of olive oils to their four origins based on multielements by yielding 92.1% and 87.3% classification and prediction rates, respectively. The classifications of olive oils to their origin is furthermore not hindered by the presence of various cultivars as the origin proved to be more significant than the cultivar in the variation of multielements concentrations in olive oils.

This work can serve as a reference for further research where olive oils representative of each Tunisian producing origin are characterized for their elemental composition to build comprehensive datasets of each producing region. This way, multielements can be applicable to help provide a scientific evidence of the uniqueness and protect the identity of oils produced inside Tunisia. In future work, correlation of the oil elemental profile with other soil parameters such as the bioavailable fraction and climatic factors need to be investigated to provide an additional proof of the usefulness of multielments as markers of the natural factors of the provenance.

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656 **Figures captions**

657 **Fig. 1.** Geological map showing the location of sampling points in Gafsa, Medenine, Sfax and
658 Monastir.

659 **Fig. 2.** LDA analysis of the element concentrations: scatterplot of the first two canonical
660 functions. (a) in olive oils using 4 groups corresponding to each origin all cultivars taken
661 together as input, (b) in olive oils using 9 groups corresponding to each single origin and
662 cultivar as input: G., Gafsa; Med., Medenine; Sf., Sfax; Mo., Monastir; Kor., Koroneiki;
663 Chemc., Chemchali; Arbos., Arbosana; Cheml., Chemlali; Frang., Frangivento and Zal.,
664 Zalmati, (c) in soils using 4 groups corresponding to each origin as input.

**Fig. 1**

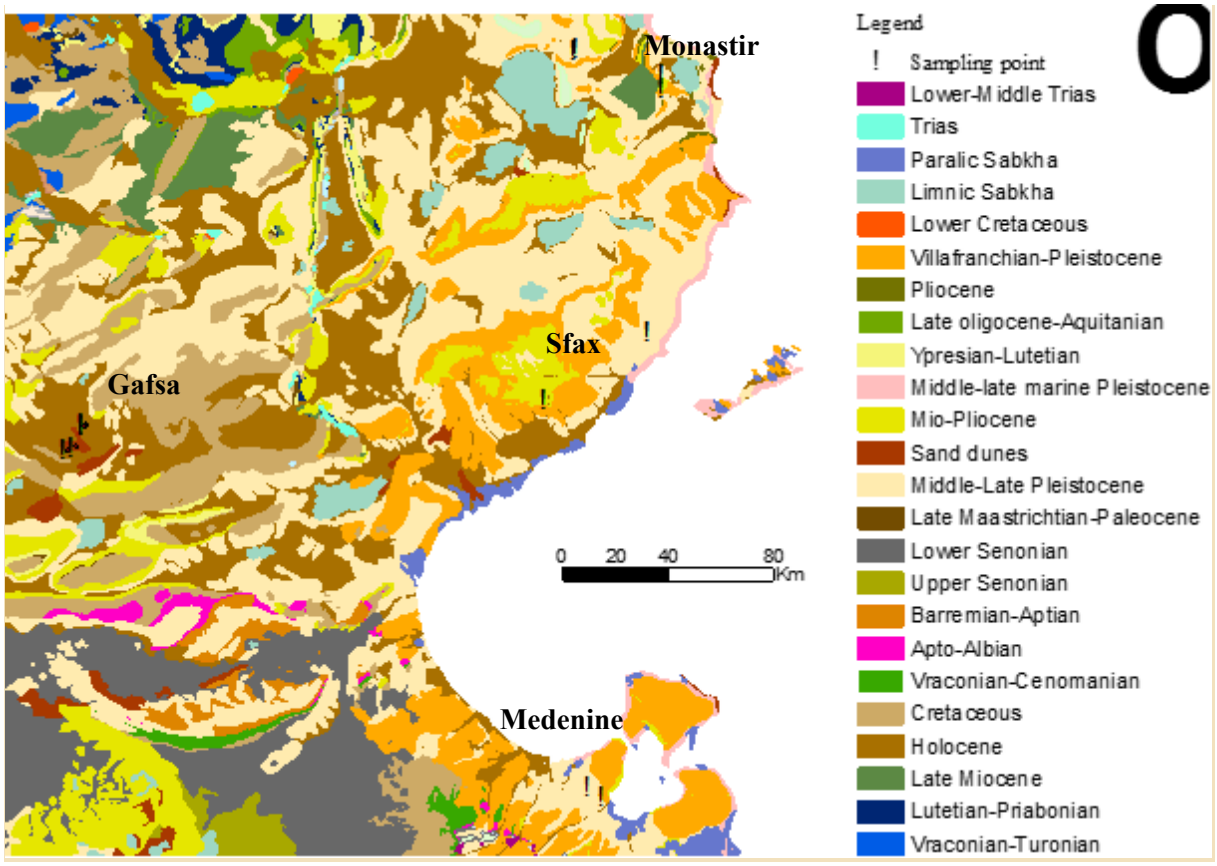
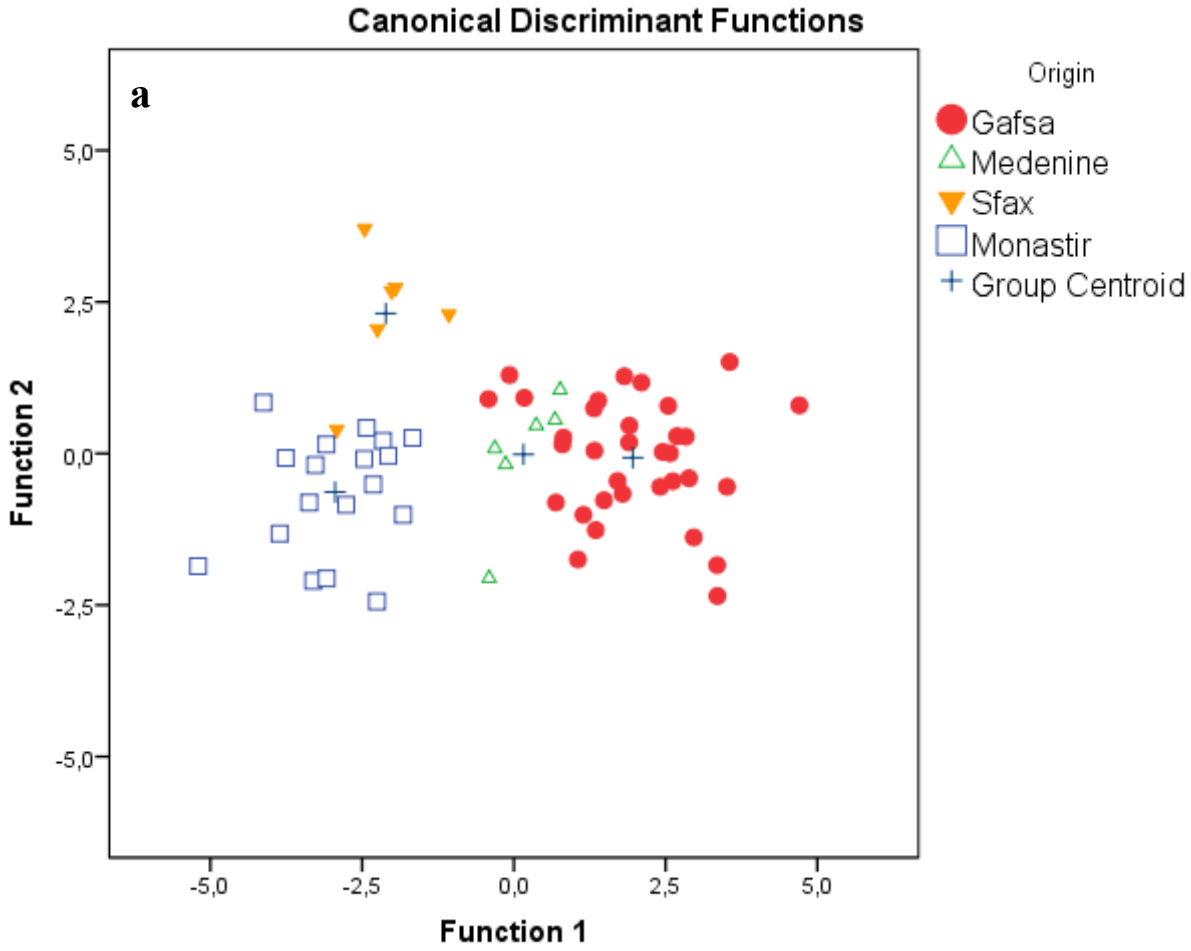
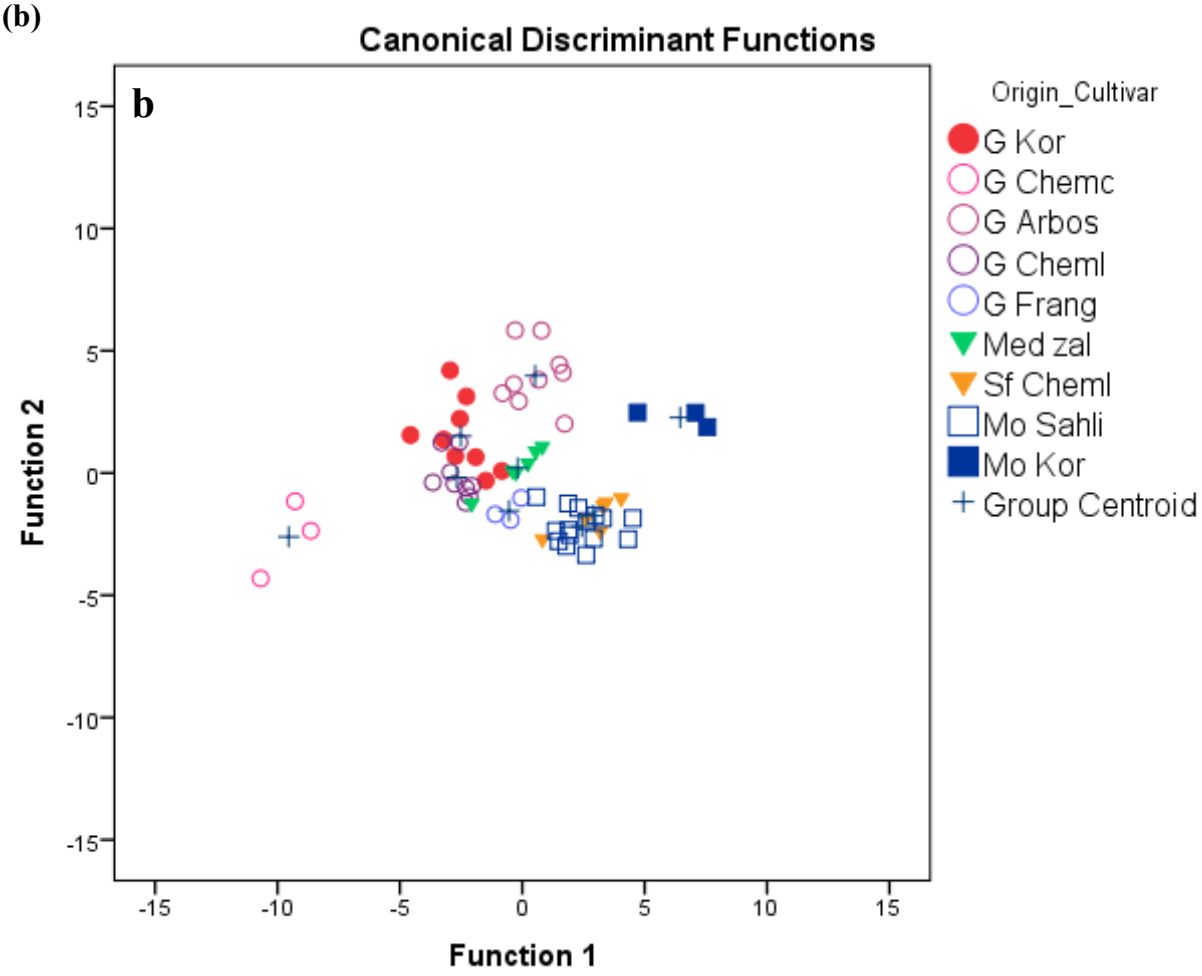

Monastir
Sfax
Gafsa
Medenine
0 20 40 80
Km
Legend
Sampling point
Lower-Middle Trias
Trias
Paralic Sabkha
Limnic Sabkha
Lower Cretaceous
Villafranchian-Pleistocene
Pliocene
Late oligocene-Aquitanian
Ypresian-Lutetian
Middle-late marine Pleistocene
Mio-Pliocene
Sand dunes
Middle-Late Pleistocene
Late Maastrichtian-Paleocene
Lower Senonian
Upper Senonian
Barremian-Aptian
Apto-Albian
Vraconian-Cenomanian
Cretaceous
Holocene
Late Miocene
Lutetian-Priabonian
Vraconian-Turonian

**Fig. 2**

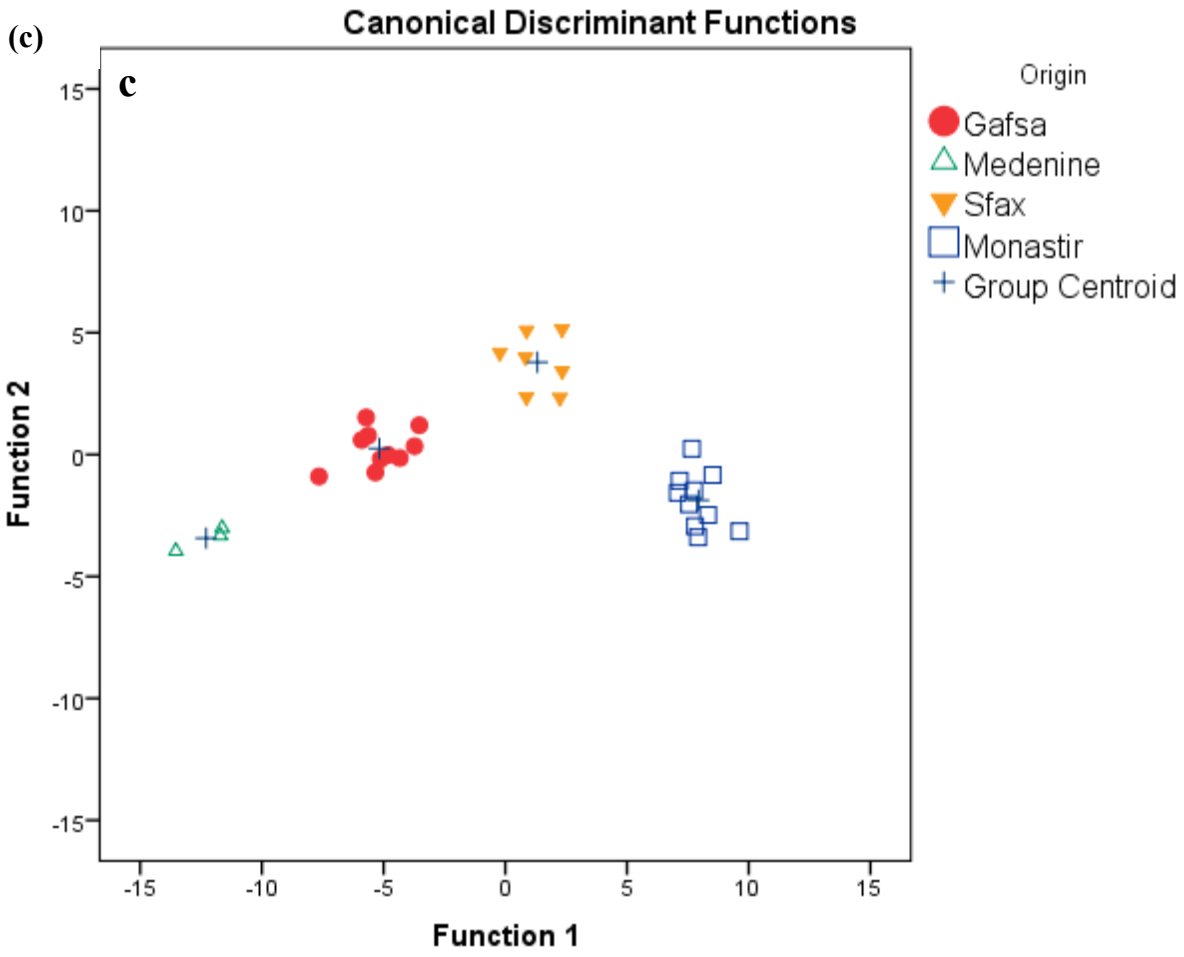
**(a)**


Canonical Discriminant Functions
a
Origin
Gafsa
Medenine
Sfax
Monastir
Group Centroid
Function 2
5,0
2,5
0,0
-2,5
-5,0
Function 1
-5,0
-2,5
0,0
2,5
5,0

**(b)**


Canonical Discriminant Functions
b
Function 2
Function 1
15
10
5
0
-5
-10
-15
-15
-10
-5
0
5
10
15
Origin_Cultivar
G Kor
G Chemc
G Arbos
G Cheml
G Frang
Med zal
Sf Cheml
Mo Sahli
Mo Kor
Group Centroid

(c)


Canonical Discriminant Functions
c
Origin
Gafsa
Medenine
Sfax
Monastir
Group Centroid
Function 2
Function 1
15
10
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-5
-10
-15
-15
-10
-5
0
5
10
15

**Table 1.** Quality control of ICP-MS analysis of olive oil samples (21 samples three times replicated).

| Element | Unit | Isotope | Operation mode | Linearity $R^2$ | Accuracy (%) | LOD | Samples > LOD (%) | LOQ | Samples > LOQ (%) |
|---|---|---|---|---|---|---|---|---|---|
| Na | mg $kg^{-1}$ | 23 | Standard | 0.9998 | 84 | 0.35 | 100 | 0.12 | 100 |
| Mg | mg $kg^{-1}$ | 24 | Standard | 0.9991 | 66 | 0.47 | 100 | 0.16 | 6 |
| Fe | mg $kg^{-1}$ | 56 | DRC | 0.9999 | 88 | 0.12 | 100 | 0.39 | 52 |
| Zn | mg $kg^{-1}$ | 66 | DRC | 0.9997 | 97 | 0.11 | 95 | 0.36 | 19 |
| V | µg $kg^{-1}$ | 51 | Standard | 1.0000 | 97 | 1.7 | 100 | 5.6 | 25 |
| Mn | µg $kg^{-1}$ | 55 | Standard | 1.0000 | 103 | 6.0 | 100 | 20 | 24 |
| As | µg $kg^{-1}$ | 75 | Standard | 0.9990 | - | 0.73 | 100 | 2.4 | 76 |
| Rb | µg $kg^{-1}$ | 85 | Standard | 0.9996 | - | 0.30 | 100 | 1.0 | 100 |
| Sr | µg $kg^{-1}$ | 88 | Standard | 0.9999 | - | 5.1 | 100 | 17 | 100 |
| Ba | µg $kg^{-1}$ | 138 | Standard | 1.0000 | 102 | 4.6 | 100 | 15 | 11 |
| Pb | µg $kg^{-1}$ | 208 | Standard | 1.0000 | 97 | 6.9 | 32 | 23 | 0 |

$R^2$: least square regression coefficient; - corresponds to elements not contained in the oil standard.

**Table 2.** Element contents in Tunisian olive oil samples from the four geographical origins independently of the cultivar.

| Element | Unit | Origin Stat. | Gafsa n=5 | Medenine n=2 | Sfax n=2 | Monastir n=5 | *p* value |
|---|---|---|---|---|---|---|---|
| Na | mg $kg^{-1}$ | Median | 4.3 a | 4.0 a | 1.6 b | 2.3 ab | *** |
| | | SD | 1.2 | 0.62 | 0.33 | 1.8 | |
| Mg | mg $kg^{-1}$ | Median | 1.2 a | 1.0 ab | 0.85 b | 0.93 ab | * |
| | | SD | 0.080 | 0.030 | 0.080 | 0.36 | |
| Fe | mg $kg^{-1}$ | Median | 0.57 | 0.43 | 0.39 | 0.28 | ns |
| | | SD | 0.49 | 0.18 | 0.15 | 0.17 | |
| Zn | mg $kg^{-1}$ | Median | 0.29 a | 0.27 ab | 0.18 bc | 0.17 c | *** |
| | | SD | 0.050 | 0.027 | 0.050 | 0.040 | |
| V | µg $kg^{-1}$ | Median | 5.2 | 5.2 | 5.1 | 4.8 | ns |
| | | SD | 0.73 | 0.50 | 1.0 | 0.81 | |
| Mn | µg $kg^{-1}$ | Median | 18 a | 13 b | 12 b | 13 b | *** |
| | | SD | 4.3 | 0.74 | 0.020 | 4.0 | |
| As | µg $kg^{-1}$ | Median | 25 ab | 29 a | 21 ab | 10 b | *** |
| | | SD | 15 | 22 | 1.9 | 3.3 | |
| Rb | µg $kg^{-1}$ | Median | 3.4 ab | 3.5 ab | 2.5 a | 3.5 b | * |
| | | SD | 0.76 | 0.040 | 0 | 3.9 | |
| Sr | µg $kg^{-1}$ | Median | 37 a | 35 ab | 33 b | 33 ab | * |
| | | SD | 2.4 | 1.3 | 1.3 | 8 | |
| Ba | µg $kg^{-1}$ | Median | 8.7 a | 9.6 ab | 12 b | 11 b | *** |
| | | SD | 0.79 | 0.10 | 0.42 | 6.0 | |
| Pb | µg $kg^{-1}$ | Median | 6.0 ab | 6.2 ab | 7.4 a | 5 b | * |
| | | SD | 1.0 | 0.90 | 0.13 | 0.96 | |

SD: standard deviation. Nonparametric Kruskal-Wallis test was applied: ns. not significant at $p > 0.05$; *. $p < 0.05$; **. $p < 0.01$; ***. $p < 0.001$. Dunn's multiple comparison adjusted by Bonferroni correction was used to make pairwise comparisons; different letters a. b. c in the same row indicate significant differences ($p < 0.05$).

**Table 3.** Loadings of PCA for element concentrations in Tunisian olive oils.

| Component | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| C.V.% | 27 | 21 | 15 | 11 |
| Rb | 0.899 | 0.059 | -0.041 | -0.212 |
| Mg | 0.789 | 0.378 | 0.144 | -0.116 |
| Sr | 0.782 | 0.196 | 0.041 | -0.089 |
| Ba | 0.716 | -0.150 | -0.298 | 0.244 |
| Mn | 0.371 | 0.835 | 0.073 | 0.001 |
| Fe | 0.074 | 0.806 | -0.326 | -0.342 |
| Zn | -0.183 | 0.629 | 0.162 | 0.280 |
| Na | 0.335 | 0.606 | 0.334 | 0.125 |
| V | 0.195 | -0.009 | 0.841 | -0.144 |
| As | -0.336 | 0.135 | 0.783 | 0.042 |
| Pb | -0.099 | 0.061 | -0.104 | 0.934 |

C.V.; Cumulative variance.

**Table 4.** Mean ± standard deviation in ppm of the element contents in soil samples from the four geographical origins at 0-30 and 30-60 cm depths.

| Element | Gafsa | | Medenine | | Sfax | | Monastir | |
|---|---|---|---|---|---|---|---|---|
| | 0-30 cm | 30-60 cm | 0-30 cm | 30-60 cm | 0-30 cm | 30-60 cm | 0-30 cm | 30-60 cm |
| Na | 1500 ± 870 | 880 ± 220 | 2000 ± 110 | 1900 ± 270 | 1100 ± 280 | 860 ± 150 | 2000 ± 860 | 1900 ± 720 |
| Mg | 3500 ± 1000 | 3600 ± 680 | 4600 ± 17 | 4400 ± 210 | 2300 ± 680 | 1700 ± 260 | 4200 ± 1400 | 4500 ± 1400 |
| Fe | 6400 ± 1700 | 6700 ± 1300 | 8600 ± 600 | 8200 ± 15 | 6000 ± 1500 | 4100 ± 300 | 14000 ± 4400 | 15000 ± 4700 |
| Zn | 18 ± 2.8 | 18 ± 3.0 | 20 ± 1.0 | 19 ± 1.6 | 23 ± 9.9 | 15 ± 0.48 | 41 ± 12 | 38 ± 7.2 |
| V | 29 ± 7.9 | 25 ± 3.6 | 34 ± 4.0 | 32 ± 7.2 | 23 ± 6.0 | 17 ± 1.3 | 45 ± 11 | 47 ± 17 |
| Mn | 84 ± 28 | 84 ± 18 | 95 ± 11 | 87 ± 0 | 79 ± 13 | 53 ± 16 | 170 ± 81 | 170 ± 76 |
| As | 2.0 ± 0.53 | 2.2 ± 0.45 | 3.2 ± 0.33 | 3.1 ± 0.13 | 2.3 ± 0.83 | 1.4 ± 0.14 | 4.2 ± 1.6 | 3.9 ± 1.2 |
| Rb | 23 ± 2.8 | 22 ± 3.3 | 34 ± 2.7 | 33 ± 0.35 | 21 ± 5.3 | 15 ± 1.7 | 40 ± 13 | 41 ± 12 |
| Sr | 110 ± 27 | 130 ± 30 | 170 ± 14 | 180 ± 2.0 | 130 ± 39 | 100 ± 12 | 140 ± 88 | 140 ± 100 |
| Ba | 190 ± 9.5 | 190 ± 16 | 290 ± 17 | 290 ± 11 | 210 ± 46 | 160 ± 17 | 270 ± 92 | 270 ± 84 |
| Pb | n.d. | n.d. | 0.17 ± 0.25 | 0.18 ± 0.25 | 0.31 ± 0.41 | n.d. | 4.4 ± 3.6 | 4.7 ± 3.7 |

n.d.; not detected.